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Interactions between leaf nitrogen status and longevity in relation to N cycling in three contrasting European forest canopies

L. Wang[1], **A. Ibrom**[2], **J. F. J. Korhonen**[3], **K. F. Arnoud Frumau**[4], **J. Wu**[2], **M. Pihlatie**[3], and **J. K. Schjoerring**[1]

[1]Plant and Soil Science Section, Department of Plant and Environmental Sciences, Faculty of Science,
University of Copenhagen Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark
[2]Technical University Denmark, Department of Chemical and Biochemical Engineering,
Centre for Ecosystem & Environmental Sustainability, Frederiksborgvej 399, Risø-Campus, 4000 Roskilde, Denmark
[3]Department of Physics, P.O. Box 48, 00014 University of Helsinki, Finland
[4]ECN, Environmental Assessment group, P.O. Box 1, 1755 ZG, Petten, The Netherlands

Correspondence to: J. K. Schjoerring (jks@life.ku.dk)

Abstract. Seasonal and spatial variations in foliar nitrogen (N) parameters were investigated in three European forests with different tree species, viz. beech (*Fagus sylvatica* L.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Scots pine (*Pinus sylvestris* L.) growing in Denmark, the Netherlands and Finland, respectively. The objectives were to investigate the distribution of N pools within the canopies of the different forests and to relate this distribution to factors and plant strategies controlling leaf development throughout the seasonal course of a vegetation period. Leaf N pools generally showed much higher seasonal and vertical variability in beech than in the coniferous canopies. However, also the two coniferous tree species behaved very differently with respect to peak summer canopy N content and N re-translocation efficiency, showing that generalisations on tree internal vs. ecosystem internal N cycling cannot be made on the basis of the leaf duration alone. During phases of intensive N turnover in spring and autumn, the NH_4^+ concentration in beech leaves rose considerably, while fully developed green beech leaves had relatively low tissue NH_4^+, similar to the steadily low levels in Douglas fir and, particularly, in Scots pine. The ratio between bulk foliar concentrations of NH_4^+ and H^+, which is an indicator of the NH_3 emission potential, reflected differences in foliage N concentration, with beech having the highest values followed by Douglas fir and Scots pine. Irrespectively of the leaf habit, i.e. deciduous versus evergreen, the majority of the canopy foliage N was retained within the trees. This was accomplished through an effective N re-translocation (beech), higher foliage longevity (fir) or both (boreal pine forest). In combination with data from a literature review, a general relationship of decreasing N re-translocation efficiency with the time needed for canopy renewal was deduced, showing that leaves which live longer re-translocate relatively less N during senescence. The Douglas fir stand, exposed to relatively high atmospheric N deposition, had by far the largest peak summer canopy N content and also returned the largest amount of N in foliage litter, suggesting that higher N fertility leads to increased turnover in the ecosystem N cycle with higher risks of losses such as leaching and gas emissions.

1 Introduction

Forests cover 44 % of the land area in Europe (FAO, 2006). Nitrogen (N) fluxes and turnover in forests are therefore relevant for biogeochemical cycling of N in Europe (Sutton et al., 2011). Forest ecosystems are generally considered to be adapted to N limitation rather than to N excess (Vitousek and Howarth, 1991; Rennenberg et al., 1998; Aerts, 1996; Xia and Wan, 2008). Due to deposition of atmospheric NH_3, the critical N load for European forest ecosystems is often exceeded (Cape et al., 2009; Pinho et al., 2012). This may in turn lead to elevated concentrations of NH_4^+ and organic N compounds in the trees and to NH_3 emission events (Rennenberg et al., 1998; Fowler et al., 2009). The physiological

drivers controlling the direction and magnitude of NH_3 exchange between trees and the atmosphere are not well established. For this reason, more information about seasonal changes in plant N status and turnover is required to improve modelling of NH_3 exchange over forest ecosystems (Massad et al., 2008, 2010).

Even under conditions with high atmospheric N deposition loads to forests, internal N fluxes between the trees and the soil (ecosystem internal cycling) and within trees (tree internal cycling) are generally larger than the external N fluxes associated with leaching and gaseous emissions (Gundersen et al., 1998; Nadelhoffer et al., 1999). Ecosystem internal N cycling takes place via processes such as litter production, mineralisation and root uptake, while tree internal N cycling occurs via assimilation, re-translocation and storage. By these processes, large amounts of N are kept plant available in the system and are being moderately protected against immobilisation in stabile soil organic compounds or losses via leaching and gaseous emission. Nitrogen in the ecosystem internal N cycle is more prone to become lost than N in the tree internal cycle, although foliar N may contribute to NH_3 emission in fertile systems under conditions with low atmospheric NH_3 concentrations (Wang et al., 2011).

Internal N re-translocation and cycling patterns in trees are seasonally programmed and closely linked to tree phenology. For instance, in deciduous trees, leaf N tends to be translocated into the woody roots and/or the trunks before leaf fall, to support tree growth in the following spring. In evergreen trees, N stored in previous-years leaves can nourish new leaf growth and, especially in most conifers, the youngest age class of needles are the main sites of N storage (Millard and Grelet, 2010). In N-limited forests, trees face a relatively high selection pressure to balance the N economy with acquisition, retention and remobilisation (Pearson et al., 2002). Knowledge about interaction between foliar N status and N re-translocation efficiency in tree species differing in leaf longevity is still limited.

It can be hypothesized that the vertical N distribution within deciduous canopies is more closely related to the actual needs for photosynthesis than is the case for longer-lived conifer needles and that the proportion of tree internal N re-translocation in general will be larger in deciduous canopies than in coniferous canopies. Along with this it will be expected that N pollution may lead to an opening of the N cycle, i.e. increase the importance of ecosystem internal over tree internal N cycling with the consequence of higher probability of N losses via leaching and gaseous emissions. On this background, the objective of the present work was to investigate the distribution of N pools within the canopies of different forests and relate this distribution to factors and plant strategies controlling leaf development throughout the seasonal course of a vegetation period. The experimental sites included a deciduous and a coniferous forest situated in strong and medium N polluted areas in temperate Europe (the Netherlands and Denmark, respectively) together with a

conifer forest in Central Finland with low N pollution. Different N parameters such as chlorophyll, N concentration per unit dry matter, C/N ratio, soluble NH_4^+, H^+, and the N content per unit projected leaf area were measured in order to obtain information on the relation to CO_2 assimilation, tree and ecosystem internal N cycling, and potential for NH_3 exchange with the atmosphere.

2 Materials and methods

2.1 Site description and sampling of plant material

The experimental sites included a Danish beech forest, a Dutch Douglas fir forest and a Finnish Scots pine forest. Details of the three localities are given in Table 1. The mean annual air temperatures in the experimental years were 9.2 and 8.6 °C for the beech forest (2008–2009); 10.2, 10.2 and 8.7 °C for the fir forest (2008–2010); and 3.2 and 3.9 °C for the pine forest (2008–2009) (Fig. 1). The corresponding precipitation values were 794 and 703 mm; 825, 735 and 780 mm; and 903 and 496 mm (Fig. 1).

It is important to note that the pollution with atmospheric N components differed substantially among the sites. The average atmospheric NH_3 concentration at the Dutch Douglas fir site was much higher (3.9 µg NH_3-N m^{-3}) than at the Danish beech forest site (1.3 µg NH_3-N m^{-3}) and at the Finnish pine site (0.1 µg NH_3-N m^{-3}). The NH_3 concentrations were measured with denuders at the sites and are annual averages from monthly integrating samples (Flechard et al., 2011). The dry deposition of total inorganic N via gasses and particles was estimated to be 30, 15 and 4 kg N ha^{-1} yr^{-1} for the forest sites in the Netherlands, Denmark and Finland, respectively (Flechard et al., 2011).

Leaves and needles were collected once or twice per month in 2008 and 2009 from the top (representing sun branches) and base (shade branches) of the crowns of 4 sample trees. In addition, monthly samples were taken in the fir forest in 2010. The needle samples were collected to obtain a representative mixture of different age classes. All plant samples were quickly weighed and frozen in liquid nitrogen upon sampling. Representative subsamples were lyophilized or oven dried at 70 °C.

2.2 Leaf analyses

Chlorophyll a (Chl a) and chlorophyll b (Chl b) concentrations in the leaves and needles were measured because these parameters are useful indicators of the physiological activity and degree of senescence. Extraction of chlorophyll was carried out by use of methanol for beech leaves, while dimethyl sulfoxide (DMSO) was used for coniferous needles. For analysis of bulk tissue NH_4^+ concentrations and pH, frozen leaves were ground in 10 mM formic acid or milli-Q water, respectively. NH_4^+ was analyzed using fluorometric detection after derivatization with o-pthaldialdehyde by FLUOstar (BMG

Table 1. Overview of site characteristics of the three investigated European forests (see alo Skiba et al., 2009).

Ecosystem	Location	Altitude (m)	Soil	Species	Annual precipitation (mm)	Annual temperature/ ($T > 10\,°C$)	Age/height of trees	Leaf area index	
Deciduous broad-leaved forest	Soro, DK (55°29′ N, 11°38′ E)	40	Oxyaquic hapludalf	Beech (*Fagus sylvatica* L.)	730	8.6 (161 d)	90 yr/25 m	4.6	Pilegaard et al. (2011)
Evergreen coniferous forests	Speulderbos, NL (52°15′ N, 5°45′ E)	52	Orthic podzol	Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco)	900	9.4 (173 d)	46 yr/32 m	5	Su et al. (2009)
	Hyytiälä, FI (61°50′ N, 24°17′ E)	181	Haplic podzol	Scots pine (*Pinus sylvestris* L)	709	2.9 (116 d)	46 yr/16 m	4.8	Launiainen (2010)

Abbreviation: DK – Denmark, NL – the Netherlands, FI – Finland.

LABTECH, Germany). For bulk tissue pH measurements, 0.2 g of leaf tissue was homogenized in a mortar on ice using 2 ml of deionized water with a little sand. The homogenate was centrifuged at 14 000 g (4 °C) for 10 min, and pH in the supernatant was measured with a microelectrode (Metrohm, Herisau, Switzerland).

The NH_3 emission potential of bulk tissue extract was estimated based on the parameter Γ, which is defined as

$$\Gamma = \frac{\left[NH_4^+\right]}{\left[H^+\right]}, \tag{1}$$

where $[NH_4^+]$ and $[H^+]$ are the NH_4^+ concentration and the proton concentration ($[H^+] = 10^{-pH}$) in the extracts, respectively.

2.3 Calculation of canopy N content and N re-translocation from senescing leaves and needles

The canopy N content (N_c), i.e. the N content of the leaves or needles per unit ground area, was calculated as the product of the measured leaf area index (I_{LAI}) and the average N content per unit projected leaf area (N_l) using measured N concentrations per unit leaf dry matter (N_{conc}) and specific leaf areas (A_{SLA}) from beech leaves (30 and 15 m^2 kg^{-1} for leaves in canopy top and base, respectively) and Douglas fir needles (79 and 54 m^2 kg^{-1} for canopy top and base, respectively), i.e.

$$N_c = N_l I_{LAI} = \frac{\overline{N_{conc}} I_{LAI}}{A_{SLA}}. \tag{2}$$

For the boreal pine stand, N_c was calculated as the product of average needle N concentration (N_{conc}; mass N per unit dry mass) obtained in the present study and estimated canopy needle dry masses (M_c, i.e. 612 g m^{-2}) that were derived from biometric relationships based on measured trunk

diameters at 1.3 m and tree heights as input (Repola, 2009):

$$N_c = \overline{N_{conc}} M_c. \tag{3}$$

The N re-translocation, R_N, in beech was estimated as the product of the measured I_{LAI} and the difference in N content per unit leaf area in summer and that of fallen leaves:

$$R_N = I_{LAI} \left(\overline{N_{l_s}} - \overline{N_{l_w}} \right). \tag{4}$$

In the conifer stands, the fallen needles were separated from the rest of the litter. The annual dry mass of green and active needles that were shed later in a year was estimated as the ratio of the measured amount of needle litter production (L) and a published mass loss factor (r_M) between green and brown needles (1/1.49, Helmisaari, 1992). This needle mass was then multiplied with the differences in N concentration between green and brown needles. From mass balance equations and L, the mass loss factor for the Dutch fir forest was estimated to be 1/1.48, i.e. very similar to that of pine.

$$R_N = \frac{L}{r_M} \left(N_{conc_s} - N_{conc_w} \right). \tag{5}$$

In the above calculations it was assumed that the average leaf N contents were sufficiently well approximated by the two layers which were investigated in this study. In summer 2005, the vertical N_l concentration profile in the experimental beech forest was investigated in relation to the cumulated leaf area above the sampled leaves. Based on this, a more complete calculation of N_c was performed. The difference between the multilayer estimate and the one using the topmost and the lowest layer as predictor for the average N_l amounted to only 1 %. The data of Meir et al. (2002) that included vertical N distributions in canopies from 5 different forest tree species were with one exception, oak, linearly related with height. Unfortunately Meir et al. (2002) did not mention the vertical leaf area distribution, and thus a similar quantitative test as above could not be performed.
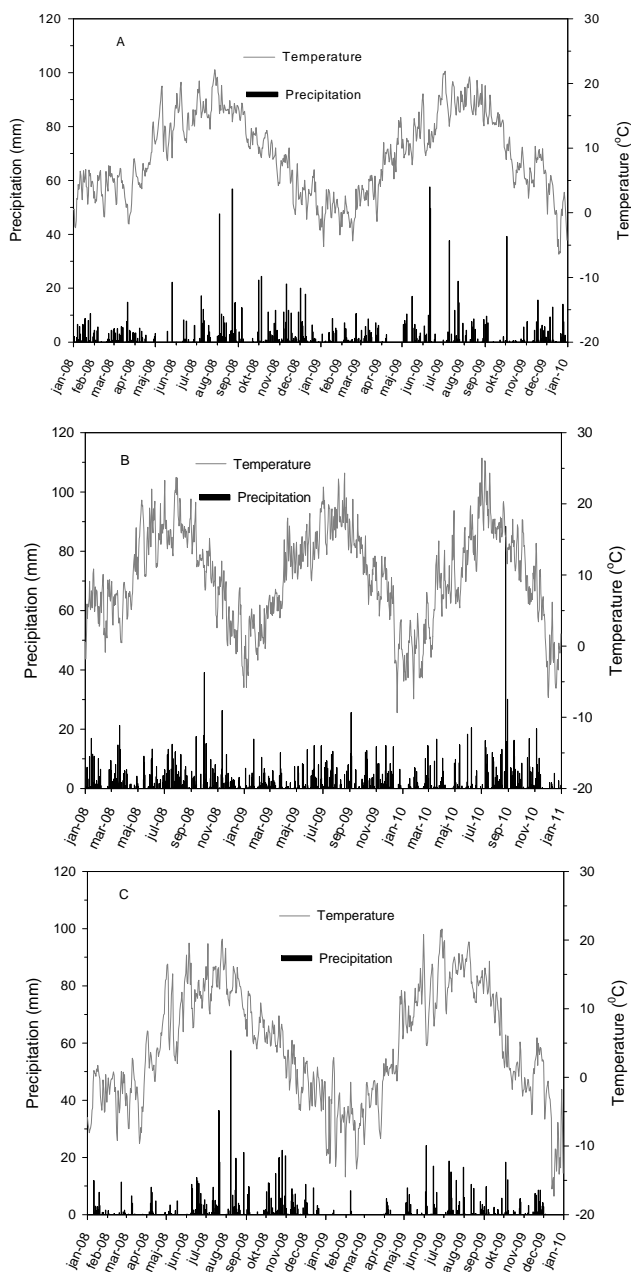
Fig. 1. Seasonal variations of air temperature (grey line) and precipitation (black columns) in the Danish beech forest from 2008 to 2009 (**A**), the Dutch Douglas fir forest from 2008 to 2010 (**B**) and the boreal Scots pine forest from 2008 to 2009 (**C**).

3 Results

3.1 Chlorophyll in leaves and needles

Both the chlorophyll concentration and the Chl a/Chl b ratio in beech increased steeply (Fig. 2-A1 and A2) during the first half of the vegetation period (May to August), far beyond the leaf expansion phase, which lasted only until June. Senescence started at the end of September and was accompanied

by a strong decrease in chlorophyll concentration and a subsequent sharp reduction of the Chl a/Chl b ratio. (Fig. 2-A2). The chlorophyll concentration as well as the Chl a/Chl b in the evergreen trees remained constant throughout the seasons (Fig. 2-B1, B2 and C1, C2). In general, the chlorophyll concentration at the canopy base was larger than that at the canopy top in both deciduous and coniferous trees.

3.2 Total nitrogen and carbon concentrations

The seasonal pattern of foliar dry matter N concentration differed among the two leaf habits (Fig. 3-A1, B1 and C1). The highest N concentrations were observed in beech leaves, peaking at about 3.5 % in very young leaves and thereupon steadily declining down to around 1 % in senescent leaves. Beech leaf senescence started earlier and the reduction in N concentration progressed faster in leaves in the top of the canopy, i.e. sun crown leaves, compared to leaves at the base, i.e. shade crown leaves (Fig. 3-A1).
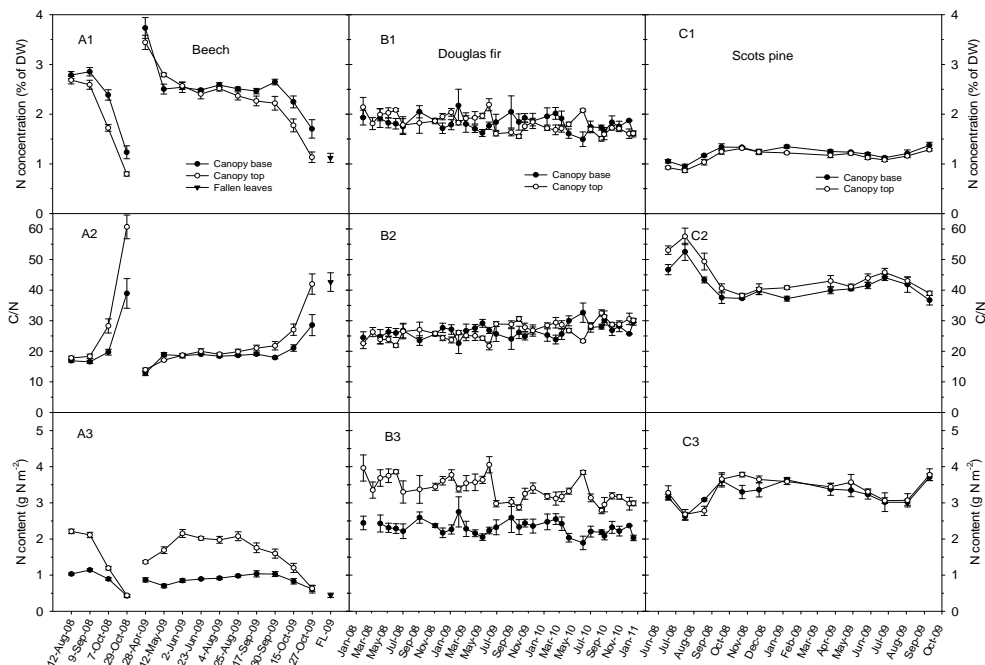
The N concentrations in Douglas fir needles did not show any seasonal trend but varied randomly between 1.5 and 2.2 %. In the Scots pine needles, the N concentration increased steadily from around 1 % to 1.3 % over the summer period and thereafter gradually declined over the following winter and spring. The beech and Scots pine canopies had slightly larger N concentrations at the canopy base compared to the top, while the Douglas fir trees had similar N concentrations in both canopy layers. The midsummer N concentration was largest in beech leaves (2.5 %) compared to Douglas fir (2.0 %) and the boreal pine (1.5 %). The mass ratio between C and N was relatively low, around 11, in very young beech leaves, but increased dramatically to above 40 during senescence, reflecting N re-translocation from the senescing beech leaves into the tree (Fig. 3-A2). Douglas fir needles had a relatively constant C/N around 25 (Fig. 3-B2, Table 2), while Scots pine needles during most of the season had values above 40 (Fig. 3-C2), i.e. more than 10 % higher than the C/N ratio in the beech leaves that were shed from the trees in the autumn. Beech leaves had the lowest N content per unit surface area and showed a progressive decline compared to needles of fir and pine, which showed little seasonal variation (Fig. 3-A3, B3 and C3). The vertical variation in beech leaves was strongest with shade leaves containing only 50 % of the N per unit leaf area compared to the sun crown leaves. Douglas fir shade needles contained 2.3 g N m^{-2}, which was 32 % less than the sun crown needles with approximately 3.4 g N m^{-2}. In contrast, the N content in pine needles (about 3.3 g N m^{-2}) was similar at both heights within the canopy.

3.3 Bulk tissue NH_4^+ and ratio between NH_4^+ and H^+ (Γ value)

Surprisingly high bulk tissue NH_4^+ concentrations, 10 mM in the top canopy leaves, were measured in very young beech leaves sampled in the canopy top just after bud break. In fully
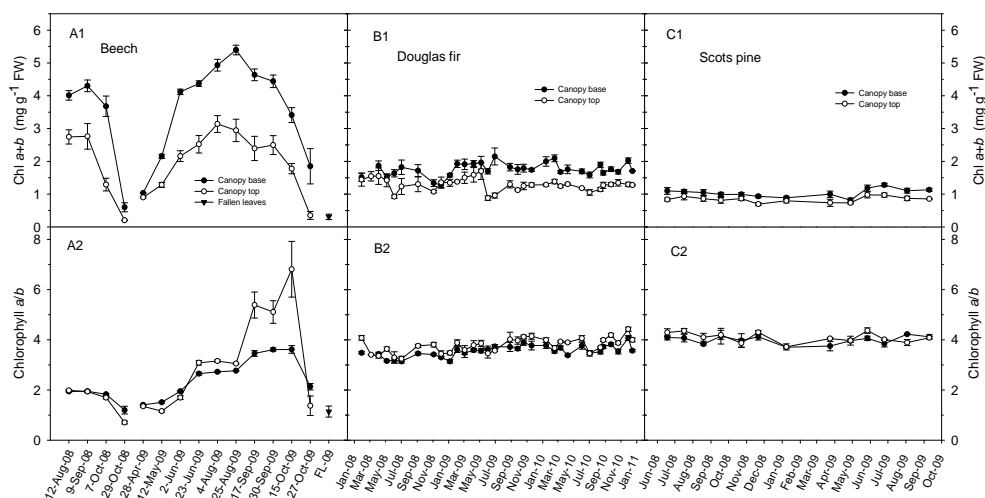
Fig. 2. Seasonal variations in leaf chlorophyll ($a + b$) concentrations and chlorophyll a/b ratio in beech leaves (A1-2), Douglas fir needles (B1-2), and Scots pine needles (C1-2).

Fig. 3. Seasonal variations in tissue N concentration per unit dry matter (upper panels), C/N ratio (mid panels) and N content per unit projected fresh leaf area (lower panels) of beech leaves (A1-3), Douglas fir needles (B1-3) and Scots pine needles (C1-3).

developed green leaves, bulk tissue NH_4^+ levels were relatively low (around 0.5–1.0 mM) but increased during senescence (Fig. 4-A1). Tissue NH_4^+ concentrations in Douglas fir needles were on average around 1.3 mM in base needles and 0.8 mM in top needles, except for the first summer period, where lower values were recorded (Fig. 4-B1). Scots pine needles had NH_4^+ concentrations around 0.4 mM, increasing to about 0.6 mM during the summer period, but showed smaller seasonal variation than the other two species (Fig. 4-C1).

Bulk tissue Γ (Fig. 4-A3, B3 and C3), i.e. the ratio between tissue concentrations of NH_4^+ (Fig. 4-A1, B1 and C1) and H^+ (Fig. 4-A2, B2 and C2), was calculated as a simple indicator for comparison of NH_3 exchange potential among the different tree species. The seasonal variation of Γ values in beech leaves paralleled that of bulk tissue H^+ rather than that of NH_4^+ concentration. Consequently, due to low pH in the young beech leaves, Γ values also turned relatively low (200–400). Beech Γ values peaked at around 400–500 during late summer, but decreased again to around 200 during

Table 2. Leaf, needle and canopy properties, N re-translocation and N litter fluxes in three different forests estimated for the year 2009.

	Sorø	Speulderbos	Hyytiälä
	Temperate European beech	Temperate Douglas fir	Boreal Scots pine
C/N in green leaves (June–August)	19	25	43
C/N in brown leaves (fallen)	38	31	103
Average leaf longevity {average time span of complete canopy renewal} (yr)	0.5 {1}	2.6 {2.6}	2.7 {2.7}
Peak summer canopy N content, N_c (g N m^{-2})	6.2	24	7.0
N flux in total aboveground litter production, F_e (g N m^{-2} yr^{-1})	7.3[a]	6.8	1.6
N flux in foliage litter production (g N m^{-2} yr^{-1})	1.8	5.8	0.8
N re-translocation by the trees, F_i (g N m^{-2} yr^{-1})	4.4	3.4	1.9
N re-translocation efficiency, η_r (% yr^{-1})[b]	70	37	71
N re-translocation by the trees per unit ground area (% yr^{-1} relative to N_c)	70	14	27
N flux in the production of new leaves (% yr^{-1} relative to N_c)	100	38	38
N uptake for the production of new leaves (% yr^{-1} relative to N_c)	30	24	11

[a] 2009 was a mast year. Fluxes were 36 % and 60 % higher than in normal years for C and N, respectively. [b] N re-translocation relative to the N content of fallen leaves when they were green in the summer.

senescence (Fig. 4-A3). This decline occurred in spite of increasing NH_4^+ concentrations due to a substantial decrease in bulk tissue pH (Fig. 4-A2).

The coniferous trees exhibited much lower Γ values than beech (Fig. 4). Douglas fir showed clear differences in Γ values between needles in the top and at the base of the canopy. The largest Γ values, fluctuating between 20 and 120, were recorded in the base needles due to higher NH_4^+ concentrations and lower H^+ (higher pH). In the top layer, Γ in Douglas fir needles consistently remained very low (<10 in most cases). Scots pine showed only moderate differences in Γ values between needles in the top or at the base of the canopy (Fig. 4-A3). In both layers, the Γ values reached a minimum of around 10 during late summer and increased to around 40 during the winter and spring.

### 3.4 Canopy nitrogen content, N re-translocation and foliar litter production

The foliage N concentration data obtained in this study were combined with field data from the investigated forest sites in order to obtain information on N cycling in the tree stands and in the ecosystems (see Sect. 2.3). Table 2 gives an overview of how the C/N ratio differed between the green and fully active leaves/needles and the brown senescent ones. The change in foliar N content was used to calculate leaf N stocks and fluxes on ecosystem scale. During senescence the C/N ratio increased by 24 % to 140 % of the summer value. The reduction in N concentration is primarily caused by tree internal N re-translocation, although other processes may potentially contribute (see further discussion in Sect. 4.3).

Very large differences between the sites were shown in the values for peak summer canopy N content (N_c, Table 2). The N content of the temperate Douglas fir canopy was 3–4 times larger than that in the two other forests, which had very similar N_c values despite their different leaf habits (Table 2).

The ratio between tree internal N cycling, i.e. N re-translocation (F_i, Table 2) and ecosystem internal N cycling, i.e. the N flux in total aboveground litter (F_e, Table 2), was calculated as 60 %, 50 % and 119 % for the beech, the fir and the pine forests, respectively. In the beech forest, intensive fruit production increased the N flux in the litter by 60 % compared to normal years. In such normal years, F_i/F_e would most probably be higher. The fraction F_i/F_e depended on the combined effects of the amount and longevity of leaves in relation to the rate of litter production from structural and reproductive plant material. All these factors differed across the investigated forests. The forest with the lowest N status had the highest F_i/F_e.

The absolute amount of N re-translocation (F_i, Table 2) was similar for the two temperate sites (4.4 and 3.4 g N m^{-2} yr^{-1}), despite their contrasting leaf habit and N_c, while the boreal site re-translocated much less (1.9 g N m^{-2} yr^{-1}). In relative terms, this picture changed completely. The fraction of N re-translocation relative to the initial N content in the green leaves, or in other words the N re-translocation efficiency (η_r), was lowest in the Douglas fir stand (37 %) but similar for the temperate beech (70 %) and the boreal pine stand (71 %). The contrasts between the sites became even more obvious when N re-translocation was related to N_c, i.e. the peak summer canopy N content, showing values of 70, 14 and 27 % for beech, Douglas fir and Scots pine, respectively (Table 2). Assuming steady state of N_c in the time scale of a few years, the proportion of N_c that was invested in new leaves during year 2009 was estimated to be 100, 38 and 38 % in beech, Douglas fir and Scots pine, respectively (Table 2). This N investment in new foliage is by
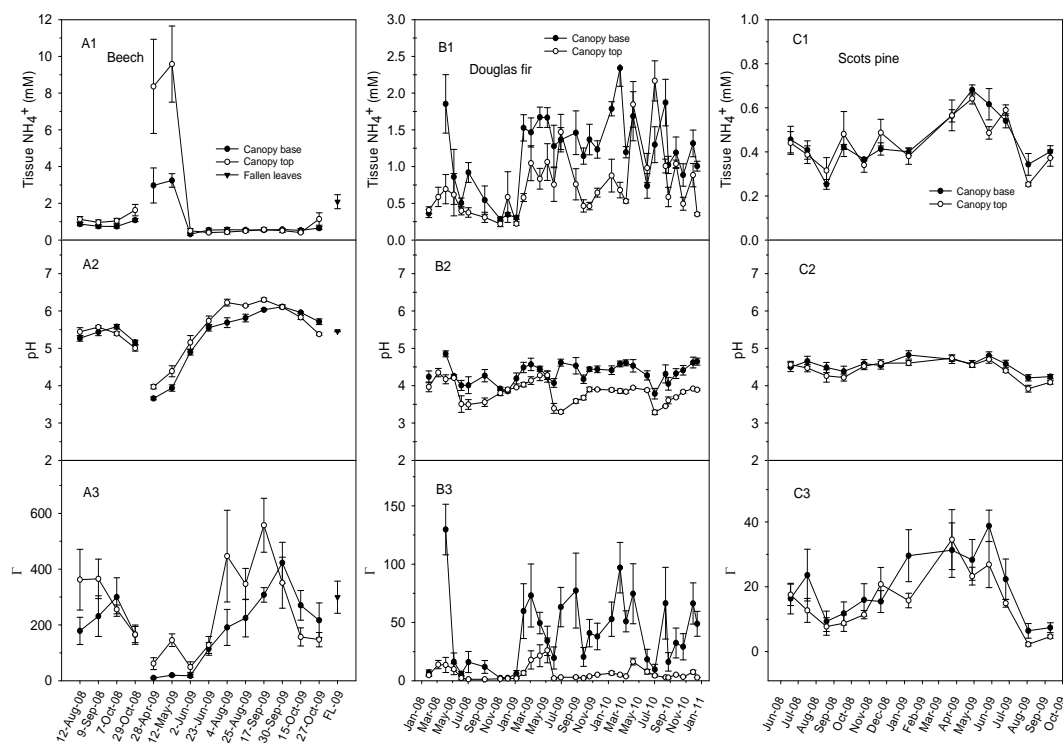
Fig. 4. Seasonal variations in bulk tissue NH_4^+ concentration, pH and tissue Γ in beech leaves (A1-3), Douglas fir needles (B1-3) and Scots pine needles (C1-3).

definition strictly related to the N_c and the average longevity of leaves or needles. A proportion of this investment is covered by formerly re-translocated N, but an additional amount needs to be taken from either external N uptake or tree internal reorganisation of N reserves. The resulting additional N uptake, which is numerically identical to the N flux in foliage litter production, ranged between 11 % and 30 % of N_c with beech having the highest and pine the lowest value (Table 2).

4 Discussion

4.1 Differences in seasonal and spatial variation of foliar N concentrations in tree canopies across the investigated forest stands

Foliar N concentrations in beech trees decreased during the spring as the leaves expanded and increased in dry matter weight over only a few weeks. During this phase, the leaf N was diluted by the build-up of C rich leaf structural material. Thereafter, the N concentrations remained constant until the onset of senescence, when N became remobilised from the leaves to perennial woody parts, leading to a rapid decrease in both the N concentrations and chlorophyll concentrations (Figs. 3-A1 and 2-A1; see also Bauer et al., 1997; Millard and Grelet, 2010). The intensive N re-translocation is necessary to conserve N for leaf establishment in spring independently

of soil N availability (Santa-Regina and Tarazona, 2001). The N re-translocation efficiency was about 55 % and 76 % for leaves at the canopy base and top of the beech trees, respectively. Similar values have been observed for beech trees at different sites in the Netherlands (Staaf, 1982) and in Japan (Yasumura et al., 2005). Beech leaves at the top and the base of the canopy started the growing season with similar dry matter N concentrations, whereupon the base leaves attained higher N concentrations during summer and autumn, reflecting higher chlorophyll concentrations (Fig. 2-A1).

Increasing chlorophyll concentrations and Chl *a*/Chl *b* ratio in beech trees during the mature green leaf period in June–August (Fig. 2-A1) reflects physiological acclimatization to increased irradiation. Chlorophyll *a* participates directly in the light harvesting reactions of photosynthesis, while chlorophyll *b* is an accessory antenna pigment acting indirectly in photosynthesis by transferring the absorbed energy to chlorophyll *a*. As the irradiation increases less antenna pigment is required, leading to increasing Chl *a*/Chl *b* ratio. Conversely, during senescence when chlorophyll is degraded and N remobilised, the core components of the photosystem seem to be more rapidly degraded leading to a sharp reduction of the Chl *a*/Chl *b* ratio (Fig. 2).

The evergreen coniferous trees showed much smaller seasonal changes in N pools, including N concentration, tissue NH_4^+ and chlorophyll concentrations compared to the deciduous beech trees (Figs. 2, 3 and 4). The analysed needle

samples included both current year needles and needles from previous years, which may have dampened seasonal changes in N pools in our observations. Minor differences between N concentrations in different age classes of Douglas fir have previously been observed at the Dutch site, older needles having slightly higher N values than young needles, as was also the case for sunlit compared to shade needles (Evers et al., 1991). Slightly higher N concentrations in older needles compared to current year needles was later confirmed by Boxman et al. (1995) and Koopmans et al. (1997). Similar differences between N concentration in young and old needles also occurred in other conifer species (Chapin and Kedrowski, 1983; Hatcher, 1990; Gebauer and Schulze, 1991; Thomas and Mead, 1992). These changes in N concentration reflect that N stored in older needles becomes remobilised to newly emerging and regrowing old needles (Millard and Grelet, 2010).

Evergreen conifers in boreal forests such as the Finnish site in the present work have evolved a conservative mechanism of nutrient turnover in order to cope with the relatively infertile soil conditions (Chabot and Hicks, 1982; Givnish, 2002). Their needles are persistent and serve as significant storage organs for carbohydrate and nutrients such as N. This storage reservoir can effectively buffer plant requirements when nutrients are not available for uptake from the soil under adverse conditions. Storage forms of N, e.g. vegetative storage proteins, are under these conditions converted to more metabolically active forms, thereby maintaining an overall relatively stable N status (Fig. 3-B1, see also Chabot and Hicks, 1982; Millard and Grelet, 2010). The re-translocation of N in Scots pine needles in Finland has been estimated to be around 73 % (Helmisaari, 1992). Across a number of evergreen shrubs and trees, the N re-translocation efficiency was on average reported to be 50 % (Aerts, 1996; Santa-Regina and Tarazona, 2001; see Fig. 5).

The higher plasticity of the N content and chlorophyll concentration in beech, even after full leaf development, compared to the coniferous trees supported our initial hypothesis that the short-lived leaves in deciduous canopies are more flexible in adjusting their functioning according to the seasonally changing weather conditions. The higher degree of shade modification in beech leaves (50 % reduced N content per unit area in shade leaves) as compared to the coniferous canopies shows that beech trees can account for vertical gradients in radiation comparably closely. There were also differences between the evergreen canopies: the fir canopy showed significant shade modification (30 %), the pine forest did not. It is obvious that the higher I_{LAI} of the fir canopy leads to stronger extinction of photosynthetically active radiation as compared to the pine forest and, consequently, shade modification is necessary to maintain productive needles in the dark canopy zones. With regard to shade modification, the leaf habit is important for the leaf structural development. In deciduous canopies, leaves can develop according to the local conditions which may vary little over the course of a
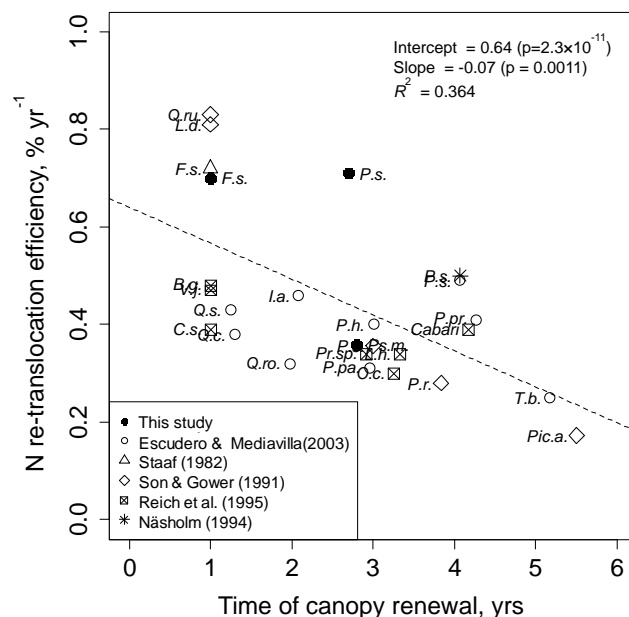
Fig. 5. Relationship between N re-translocation efficiency and time needed for complete foliage canopy renewal in the present and other studies. Abbreviated plant taxa: *B.g.: Bellucia grossularioides*, *C.s.: Clidemia serice, Cabari: Cabari, F.s.: Fagus sylvatica, I.a.: Ilex aquifolium, L.d.: Larix decidua, L.h.: Licania heteromorpha, O.c.: Ocotea costulata, P.h.: Pinus halepensis, P.pa.: Pinus pinea, P.pr.: Pinus pinaster, P.r.: Pinus resinosa, P.s.: Pinus sylvestris, P.s.: Pinus strobus, Pic.a.: Picea abies, Pr.sp.: Protium sp., Ps.m.: Pseudotsuga menziesii, Q.c.: Quercus coccifera, Q.ro.: Quercus rotundifolia, Q.ru.: Quercus rubra, Q.s.: Quercus suber, T.b.: Taxus baccata,* and *V.j.: Vismia japurensis.* The canopy renewal time for *P.s.* needles in Näsholm (1994) was assumed to be the same as that for *P.s.* in Escudero and Mediavilla (2003).

year. In conifer canopies, leaves live longer and thus their relative position in the crown can change due to growth of new branches in the top. Nitrogen economy can lead to transient physiological modification and to transient shading, but needle structures stay more or less the same. This might set limits on shade modification in dense growing conifer canopies compared to deciduous canopies.

4.2 Seasonal variations in bulk tissue Γ

A useful temperature-normalized parameter for evaluation and modelling of NH_3 exchange between plant leaves and the atmosphere is the apoplastic Γ value (Γ_{apo}), which is equal to the ratio between $[NH_4^+]$ and $[H^+]$ in the leaf apoplastic solution (Nemitz et al., 2000; Mattsson and Schjoerring, 2002; Massad et al., 2010; Zhang et al., 2010). In the present work it was not possible to obtain apoplastic parameters. Instead, the ratio between bulk tissue concentrations of NH_4^+ and H^+ was measured. There are indications that bulk tissue values may be important bioindicators for N turnover processes, particularly during incipient senescence

where the tissue concentration of NH_4^+ may increase in parallel in both leaf tissue and apoplastic solution (Mattsson and Schjoerring, 2003). There is also evidence that bulk leaf litter NH_4^+ and H^+ concentrations in senescent tissues may be more important for the NH_3 exchange than the corresponding apoplastic concentrations (Husted et al., 2000; Nemitz et al., 2000). Bulk tissue values therefore still have some relevance for characterization of nitrogen pools and their relation to overall nitrogen dynamics during the growth season.

The largest bulk tissue Γ values (Γ_{bulk}) were 500 in beech leaves, while in most cases Γ_{bulk} values in Douglas fir and Scots pine needles remained below 75 and 40, respectively (Fig. 4). Obviously, the relative differences between the three canopies were not directly related to the atmospheric NH_3 concentration or the dry deposition of total inorganic N at the three sites, which on average were 3.9, 1.3 and 0.1 µg NH_3-N m^{-3} or 30, 15 and 4 kg N ha^{-1} yr^{-1} at the Dutch Douglas fir site, the Danish beech forest site and the Finish Scots pine site, respectively (see Sect. 2.1). Such a relationship would not be anticipated because tissue NH_4^+ relations are affected by a range of species-dependent physiological processes involved in N uptake and N turnover in the leaves (Schjoerring et al., 2000). Rather than differences in atmospheric concentrations, the Γ_{bulk} values therefore reflect foliage N status, in agreement with that beech leaves had the highest N concentration per unit leaf mass, followed by Douglas fir and Scots pine (Fig. 3). The Γ_{bulk} values measured in the three tree species were in all cases much lower than those measured previously in grass species (Kruit et al., 2007; David et al., 2009; Mattsson et al., 2009).

The question is to what an extent Γ_{bulk} can be used as a proxy for the NH_3 exchange potential of plant leaves. For the coniferous species, the Γ_{bulk} values were considerably lower than previously reported Γ_{apo} values as reviewed by Zhang et al. (2010). A clear seasonal trend in Γ_{bulk} values was not observed for the coniferous species in the present work (Fig. 4). In contrast, Γ_{apo} values in Scots pine needles in a mixed coniferous forest subjected to high N deposition were relatively high (3300) during spring and declined to 1375 during the summer/autumn (Neirynck and Ceulemans, 2008). The difference in seasonal variations between Γ_{bulk} and Γ_{apo} may reflect that NH_4^+ and H^+ concentrations are better regulated in the bulk tissue than in the apoplastic solution. In beech leaves, Γ_{bulk} was very low during leaf development, increased during the mature green leaf period, and declined again during leaf senescence (Fig. 4). This pattern did not match the results derived from the NH_3 flux measurement, showing the lowest NH_3 emission during the green leaf period (Wang et al., 2011). Further gas exchange measurement and bioassays of the apoplastic solution in coniferous needles are needed to evaluate the possibility of substituting Γ_{apo} with the simpler Γ_{bulk} value.

The strong seasonality of the NH_4^+ concentration in beech leaves points to a surprising relationship between Γ_{bulk} values and N re-translocation. In phases when leaves were either expanding or started to senesce the NH_4^+ concentration increased in response to the biochemical reactions involved in the N turnover (Fig. 4; Schjoerring et al., 2000; Britto and Kronzucker, 2002). However, the Γ_{bulk} values were even lower during these periods, because the bulk tissue pH values decreased rapidly (Fig. 4). This pH drop can be interpreted as a so far unconsidered physiological N conservation mechanism in beech leaves counteracting gaseous NH_3 losses. The discussion of the relationships between cellular N transformations and proton production/consumption is beyond the scope of this article.

4.3 Other effects than N re-translocation that might have been contributed to decreasing N contents in leaves and needles

In agreement with many other studies, we interpreted the strong reduction in leaf N content as N re-translocation (May and Killingbeck, 1992; Killingbeck, 1996; Escudero et al., 2003), while we neglected NH_3 emission and N leaching from senescing leaves and needles as factors involved in N content reduction. As explained above, the contribution of NH_3 emission to the N loss in the leaves and needles during senescence could be excluded, because the measured bulk tissue Γ values were too small to build up a sufficiently high NH_3 partial pressure in the leaves. Possible leaching losses from fallen leaves or needles were minimised by fast sampling and analysis, while leaching losses from senescing leaves or needles in the canopies were not investigated. If leaching was a substantial process, it would cause a strong N enrichment in the rain underneath the canopy. However, such enrichment was not recorded in e.g. the beech forest where canopy throughfall measurements in 1998–1999 showed that the annual sum of throughfall N on average was 0.66 g N m^{-2} with monthly rates during September and October ranging between 0.02 and 0.25 g N m^{-2} (Beier et al., 2001). This amount of N only constituted approx. 6 % of the estimated N re-translocation measured over one to two months in our study. We cannot entirely exclude effects of N leaching or NH3 volatilisation from senescing leaves, but given these considerations the contribution would very likely be small.

4.4 Relationships between nitrogen cycling, leaf habit and N status of the ecosystem

We expected that producing every year an entirely new canopy or not should have large implications for N cycling in forest trees and ecosystems and thus expected that the leaf habit, deciduous vs. evergreen, would be a major determinant for the internal N turnover in forest ecosystems. The aboveground litter production is a dominant component of the ecosystem internal N cycle, and as long as N is cycling within the trees it is protected against losses *via* leaching (NO_3^-), volatilisation (NO, N_2O, N_2, NH_3) and soil immobilisation (NH_4^+ fixation or formation of stable N containing

soil organic matter compounds). Trees can and do control how much N that will cycle in the ecosystem and how much that will be stored within the trees. Our results show that the investigated deciduous forest supplied the ecosystem internal N cycle with only $1.8\,\mathrm{g\,N\,m^{-2}\,yr^{-1}}$ through leaf litter production compared to $5.8\,\mathrm{g\,N\,m^{-2}\,yr^{-1}}$ in the temperate evergreen Douglas fir forest (Table 2), pointing to strong differences between trees with different leaf habit. However, our results also show that evergreen is not evergreen, because the N flux in needle litter in the boreal pine forest, $0.8\,\mathrm{g\,N\,m^{-2}}$ $\mathrm{yr^{-1}}$ (Table 2), only constituted 14 % of that of the temperate Douglas fir stand. The key difference between the two coniferous forest canopies was the canopy summer N content (N_c) which was 3.4 times larger in the temperate Douglas fir stand than in the boreal pine stand (Table 2). The N re-translocation per unit leaf area of the pine trees reached the same efficiency as that of the deciduous forest (ca. 70 %), whereas the Douglas fir stand had a re-translocation efficiency of only 37 %, meaning that it relocated only 53 % as efficiently as the two other species (Table 2). Inclusion of data on the N re-translocation efficiency from previously published studies (Staaf, 1982; Son and Gover, 1991; Näsholm et al., 1994; Reich et al., 1995; Escudero and Mediavilla, 2003) revealed that the longer the needles stayed green, the lower the N re-translocation efficiency (η_r) was (Fig. 5). This can be interpreted as a functional adaptation against N losses in trees with short-living leaves or needles, i.e. frequent need for canopy renewal. Despite large variability especially between temperate vs. Mediterranean deciduous trees, the data still showed a general significant linear decrease in η_r with increasing canopy renewal time (7 % less N re-translocated per year the canopy stayed longer; Fig. 5). A simple distinction between deciduous and evergreen mode is thus not sufficient to generalise N re-translocation efficiencies. The leaf habit is one factor among others. The two coniferous sites showed that the canopy needle mass and its N content must be taken into account together with differences in the way the evergreen forests renew their canopies and save N via re-translocation. The results from the three forest sites suggest that some of these processes are not independent from each other; the efficiency with which the N is re-translocated before leaf fall (η_r) and the time needed to renew the canopy, mainly determined by leaf longevity, were related. Because of species specific differences, we recommend investigating the relationship between η_r and canopy renewal time with a larger data set of forests and possibly by inclusion of other natural and semi-natural systems.

Another explanation for the observed differences in N re-translocation efficiencies could be that the high N availability at the Douglas fir site might have led to the reduced N re-translocation, because, for example, tree internal buffers for N reserves were already filled. The canopy N content reflects the N status of the system. Higher air concentrations of reactive N compounds (e.g. NH_3) and N deposition loads together with a more productive climate enable the establishment of a larger and more fertile canopy in the Douglas fir stand. The effective N re-translocation in the boreal pine forest supports a possible distinction between N scarce and N rich evergreen ecosystems, leading to a more strict N economy, i. e. reduced ecosystem internal N cycling as our initial hypothesis stated.

Summarising the results from this study, we show that forests can realise very different mechanisms to keep N losses in foliage litter production low: (i) increased N re-translocation efficiency, (ii) increased leaf longevity, and (iii) decreased canopy nitrogen content. Despite having the shortest leaf longevity and highest leaf N concentration per unit dry matter, the beech canopy reduced the N losses via leaf litter production with very efficient N re-translocation and established only a small amount of leaf mass with a small N content per unit ground. Compared to the beech stand, the N scarce boreal pine forest carried an over twice as high foliage mass, which was renewed on average every 2.7 yr with a very low N concentration per unit dry matter and a low canopy N content. Together with equally efficient N re-translocation, the N losses to the soil via foliage litter production were lowest in the boreal pine forest. The temperate N-rich Douglas fir stand had a fairly high foliar N concentration, established the by far highest foliage mass and canopy N content, and had the lowest N re-translocation efficiency. The only mechanism that kept the foliage N in the Douglas fir trees was the longevity of the needles

Mechanistic models that aim to represent the dynamics of carbon and N pools and budgets need to include these factors and their relative contribution to conserve the N in forest trees. In general, the N dynamics modules require information about the N supply in the soil, N uptake demand of the trees, N losses and also the N re-translocation before litter fall, e.g. Jansson and Karlberg (2004) and Zhang et al. (2006), where η_r, i.e. the N re-translocation efficiency, is a necessary input parameter. For realistic modelling, the canopy specific differences and possible interactions between N status and N re-translocation need to be taken into account. The indicated relationship between η_r and canopy renewal time might help to describe systematic variation in η_r across forests.

5 Conclusions

Leaf N pools showed clear seasonal changes in beech trees while remaining relatively constant in the evergreen canopies, i.e. Douglas fir and Scots pine. The ratio between bulk tissue concentrations of NH_4^+ and H^+) (Γ_{bulk}) did not directly relate to the atmospheric NH_3 concentrations or N deposition loads at the three sites, but it reflected plant N status. This is in agreement with the fact that beech leaves had the highest N concentration, followed by Douglas fir and Scots pine. Irrespectively of the leaf habit, all investigated trees retained the majority of the canopy foliage N in

the trees. This was done through effective N re-translocation (beech), higher foliage longevity (fir) or both (boreal pine forest). A simple generalisation of the mechanisms of the tree internal N cycling based on the leaf habit could not be made; the differences between the two investigated evergreen forests were as large as those measured relative to the deciduous canopy. Efficient N re-translocation is not restricted to deciduous canopies but can also be found in the N-limited forests, which released the lowest amount of N to the soil via foliage litter and to ecosystem internal cycling in this study. Apart from the efficiency of N re-translocation, the amount of N in the canopy and the fraction of annual leaf renewal are important determinants for the N turnover in trees and the contribution of N uptake from and release to the ecosystem internal N cycle. Our results indicate that there may be a general relationship of decreasing N re-translocation efficiency with the time needed for canopy renewal, i.e. leaves that live longer re-translocate relatively less N during senescence. In the stand with the highest atmospheric N deposition, i.e. the Douglas fir stand, the canopy N content and the flux rate of N in foliage litter production was largest, leading to higher turnover in the ecosystem N cycle and implying higher risks of N losses via leaching and gaseous emissions.

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References

Aerts, R.: Nutrient resorption from senescing leaves of perennials: Are there general patterns?, J. Ecol., 84, 597–608, 1996.

Bauer, G., Schulze, E. D., and Mund, M.: Nutrient contents and concentrations in relation to growth of Picea abies and Fagus sylvatica along a European transect, Tree Physiol., 17, 777–786, 1997.

Beier, C., Rasmussen, L., Pilegaard, K., Ambus, P., Mikkelsen, T., Jensen, N. O., Kjøller, A., Priemé, A., and Ladekarl, U. L.: Fluxes of NO_3^-, NH_4^+, NO, NO_2, and N_2O in an old Danish beech forest, Water Air Soil Pollut. Focus, 1, 187–195, 2001.

Boxman, A. W., Van Dam, D., Van Dijk, H. F. G., Hogervorst, R. F., and Koopmans, C. J.: Ecosystem responses to reduced nitrogen and sulphur inputs into two coniferous forest stands in the Netherlands, Forest Ecol. Manag., 71, 7–29, 1995.

Britto, D. T. and Kronzucker, H. J.: NH_4^+ toxicity in higher plants: a critical review, J. Plant Physiol., 159, 567–584, 2002.

Cape, J. N., van der Eerden, L. J., Sheppard, L. J., Leith, I. D., and Sutton, M. A.: Evidence for changing the critical level for ammonia, Environ. Pollut., 157, 1033–1037, 2009.

Chabot, B. F. and Hicks, D. J.: The ecology of leaf life span, Annu. Rev. Ecol. Systemat., 13, 229–259, 1982.

Chapin, F. S. and Kedrowski, R. A.: Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees, Ecology, 64, 376–391, 1983.

David, M., Loubet, B., Cellier, P., Mattsson, M., Schjoerring, J. K., Nemitz, E., Roche, R., Riedo, M., and Sutton, M. A.: Ammonia sources and sinks in an intensively managed grassland canopy, Biogeosciences, 6, 1903–1915, doi:10.5194/bg-6-1903-2009, 2009.

Escudero, A., Iriondo, J. M., and Torres, M. E.: Spatial analysis of genetic diversity as a tool for plant conservation, Biol. Conservat., 113, 351–365, 2003.

Escudero, A. and Mediavilla, S.: Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf life span, J. Ecol., 91, 880–889, 2003.

Evers, P. W., Jans, W. W. P., and Steingröver, E. G.: Impact of air pollution on ecophysiological relations in two Douglas fir stands in the Netherlands. De Dorschkamp, Research Institute for Forestry and Urban Ecology, Wageningen, Rep. no. 637, 306 pp., 1991.

FAO: Global forest resources assessment 2005. Progress towards sustainable forest management, FAO Forestry paper 147, 11–36, 2006.

Flechard, C. R., Nemitz, E., Smith, R. I., Fowler, D., Vermeulen, A. T., Bleeker, A., Erisman, J. W., Simpson, D., Zhang, L., Tang, Y. S., and Sutton, M. A.: Dry deposition of reactive nitrogen to European ecosystems: a comparison of inferential models across the NitroEurope network, Atmos. Chem. Phys., 11, 2703–2728, doi:10.5194/acp-11-2703-2011, 2011.

Fowler, D., Pilegaard, K., Sutton, M. A., Ambus, P., Raivonen, M., Duyzer, J., Simpson, D., Fagerli, H., Fuzzi, S., Schjoerring, J. K., Granier, C., Neftel, A., Isaksen, I. S. A., Laj, P., Maione, M., Monks, P. S., Burkhardt, J., Daemmgen, U., Neirynck, J., Personne, E., Wichink-Kruit, R., Butterbach-Bahl, K., Flechard, C., Tuovinen, J. P., Coyle, M., Gerosa, G., Loubet, B., Altimir, N., Gruenhage, L., Ammann, C., Cieslik, S., Paoletti, E., Mikkelsen, T. N., Ro-Poulsen, H., Cellier, P., Cape, J. N., Horvath, L., Loreto, F., Niinemets, U., Palmer, P. I., Rinne, J., Misztal, P., Nemitz, E., Nilsson, D., Pryor, S., Gallagher, M. W., Vesala, T., Skiba, U., Brueggemann, N., Zechmeister-Boltenstern, S., Williams, J., O'Dowd, C., Facchini, M. C., de Leeuw, G., Flossman, A., Chaumerliac, N., and Erisman, J. W.: Atmospheric composition change: Ecosystems-Atmosphere interactions, Atmos. Environ., 43, 5193–5267, 2009.

Gebauer, G. and Schulze, E. D.: Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria, Oecologia, 87, 198–207, 1991.

Givnish, T. J.: Adaptive significance of evergreen vs. deciduous leaves: Solving the triple paradox, Silva Fennica, 36, 703–743, 2002.

Gundersen, P., Emmett, B. A., Kjonaas, O. J., Koopmans, C. J., and Tietema, A.: Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data, Forest Ecol. Manag., 101, 37–55, 1998.

Hatcher, P. E.: Seasonal and age-related variation in the needle quality of five conifer species, Oecologia, 85, 200–212, 1990.

Helmisaari, H. S.: Nutrient retranslocation within the foliage of *Pinus sylvestris*, Tree Physiol., 10, 45–58, 1992.

Husted, S., Schjoerring, J. K., Nielsen, K. H., Nemitz, E., and Sutton, M. A.: Stomatal compensation points for ammonia in oilseed rape plants under field conditions, Agric. Forest Meteorol., 105, 371–383, 2000.

Jansson, P. and Karlberg, L.: Coup manual-coupled heat and mass transfer model for soil-plantatmosphere systems, Technical manual for the CoupModel, 2004.

Killingbeck, K. T.: Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency, Ecology, 77, 1716–1727, 1996.

Koopmans, C. J., vanDam, D., Tietema, A., and Verstraten, J. M.: Natural ^{15}N abundance in two nitrogen saturated forest ecosystems, Oecologia, 111, 470–480, 1997.

Kruit, R. J. W., van Pul, W. A. J., Otjes, R. P., Hofschreuder, P., Jacobs, A. F. G., and Holtslag, A. A. M.: Ammonia fluxes and derived canopy compensation points over non-fertilized agricultural grassland in The Netherlands using the new gradient ammonia – high accuracy – monitor (GRAHAM), Atmos. Environ., 41, 1275–1287, 2007.

Launiainen, S.: Seasonal and inter-annual variability of energy exchange above a boreal Scots pine forest, Biogeosciences, 7, 3921–3940, doi:10.5194/bg-7-3921-2010, 2010.

Massad, R. S., Loubet, B., Tuzet, A., and Cellier, P.: Relationship between ammonia stomatal compensation point and nitrogen metabolism in arable crops: Current status of knowledge and potential modelling approaches, Environ. Pollut., 154, 390–403, 2008.

Massad, R. S., Tuzet, A., Loubet, B., Perrier, A., and Cellier, P.: Model of stomatal ammonia compensation point (STAMP) in relation to the plant nitrogen and carbon metabolisms and environmental conditions, Ecol. Modell., 221, 479–494, 2010.

Mattsson, M. and Schjoerring, J. K.: Dynamic and steady-state responses of inorganic nitrogen pools and NH_3 exchange in leaves of *Lolium perenne* and *Bromus erectus* to changes in root nitrogen supply, Plant Physiol., 128, 742–750, 2002.

Mattsson, M. and Schjoerring, J. K.: Senescence-induced changes in apoplastic and bulk tissue ammonia concentrations of ryegrass leaves, New Phytol., 160, 489–499, 2003.

Mattsson, M., Herrmann, B., David, M., Loubet, B., Riedo, M., Theobald, M. R., Sutton, M. A., Bruhn, D., Neftel, A., and Schjoerring, J. K.: Temporal variability in bioassays of the stomatal ammonia compensation point in relation to plant and soil nitrogen parameters in intensively managed grassland, Biogeosciences, 6, 171–179, doi:10.5194/bg-6-171-2009, 2009.

May, J. D. and Killingbeck, K. T.: Effects of preventing nutrient resorption on plant fitness and foliar nutrient dynamics, Ecology, 73, 1868–1878, 1992.

Meir, P., Kruijt, B., Broadmeadow, M., Barbosa, E., Kull, O., Carswell, F., Nobre, A., and Jarvis, P. G.: Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. Plant, Cell Environ., 25, 343–357, 2002.

Millard, P. and Grelet, G. A.: Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world, Tree Physiol., 30, 1083–1095, 2010.

Nadelhoffer, K. J., Emmett, B. A., Gundersen, P., Kjonaas, O. J., Koopmans, C. J., Schleppi, P., Tietema, A., and Wright, R. F.: Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests, Nature, 398, 145–148, 1999.

Neirynck, J. and Ceulemans, R.: Bidirectional ammonia exchange above a mixed coniferous forest, Environ. Pollut., 154, 424–438, 2008.

Nemitz, E., Sutton, M. A., Schjoerring, J. K., Husted, S., and Wyers, G. P.: Resistance modelling of ammonia exchange over oilseed rape, Agr. Forest Meteorol., 105, 405–425, 2000.

Näsholm, T.: Removal of nitrogen during needle senescence in Scots pine (*Pinus sylvestris* L.), Oecologia, 99, 290–296, 1994.

Pearson, J., Woodall, J., Clough, E. C. M., Nielsen, K. H., and Schjoerring, J. K.: Production and consumption of NH_3 in trees, in: Trace gas exchange in forest ecosystems, edited by: Gasche, R., Papen, H., and Rennenberg, H., Kluwer Academic Publishers, The Netherlands, 53–77, 2002.

Pilegaard, K., Ibrom, A., Courtney, M. S., Hummelshoj, P., and Jensen, N. O.: Increasing net CO_2 uptake by a Danish beech forest during the period from 1996 to 2009, Agr. Forest Meteorol., 151, 934–946, 2011.

Pinho, P., Theobald, M. R., Dias, T., Tang, Y. S., Cruz, C., Martins-Loução, M. A., KMáguas, C., Sutton, M., and Branquinho, C.: Critical loads of nitrogen deposition and critical levels of atmospheric ammonia for mediterranean evergreen woodlands, Biogeosciences, 9, 1205–1215, doi:10.5194/bg-9-1205-2012, 2012.

Reich, P. B., Ellsworth, D. S., and Uhl, C.: Leaf carbon and nutrient assimilation and conservation in species of differing successional Status in an oligotrophic Amazonian forest, Funct. Ecol., 9, 65–76, 1995.

Rennenberg, H., Kreutzer, K., Papen, H., and Weber, P.: Consequences of high loads of nitrogen for spruce (*Picea abies*) and beech (*Fagus sylvatica*) forests, New Phytol., 139, 71–86, 1998.

Repola, J.: Biomass equations for Scots pine and Norway spruce in Finland, Silva Fennica, 43, 625–647, 2009.

Santa-Regina, I. and Tarazona, T.: Nutrient cycling in a natural beech forest and adjacent planted pine in northern Spain, Forestry, 74, 11–28, 2001.

Schjoerring, J. K., Husted, S., Mack, G., Nielsen, K. H., Finnemann, J., and Mattsson, M.: Physiological regulation of plant-atmosphere ammonia exchange, Plant Soil, 221, 95–102, 2000.

Skiba, U., Drewer, J., Tang, Y. S., van Dijk, N., Helfter, C., Nemitz, E., Famulari, D., Cape, J. N., Jones, S. K., Twigg, M., Pihlatie, M., Vesala, T., Larsen, K. S., Carter, M. S., Ambus, P., Ibrom, A., Beier, C., Hensen, A., Frumau, A., Erisman, J. W., Bruggemann, N., Gasche, R., Butterbach-Bahl, K., Neftel, A., Spirig, C., Horvath, L., Freibauer, A., Cellier, P., Laville, P., Loubet, B., Magliulo, E., Bertolini, T., Seufert, G., Andersson, M., Manca, G., Laurila, T., Aurela, M., Lohila, A., Zechmeister-Boltenstern, S., Kitzler, B., Schaufler, G., Siemens, J., Kindler, R., Flechard, C., and Sutton, M. A.: Biosphere-atmosphere exchange of reactive nitrogen and greenhouse gases at the NitroEurope core flux measurement sites: Measurement strategy and first data sets, Agr. Ecosyst. Environ., 133, 139–149, 2009.

Son, Y. and Gower, S. T.: Aboveground nitrogen and phosphorus use by five plantation-grown trees with different leaf longevities, Biogeochemistry, 14, 167–191, 1991.

Staaf, H.: Plant nutrient changes in beech leaves during senescence as influenced by site characteristics, Acta Oecologica-Oecologia Plantarum, 3, 161–170, 1982.

Su, Z., Timmermans, W. J., van der Tol, C., Dost, R., Bianchi, R., Gómez, J. A., House, A., Hajnsek, I., Menenti, M., Magliulo, V., Esposito, M., Haarbrink, R., Bosveld, F., Rothe, R., Baltink, H. K., Vekerdy, Z., Sobrino, J. A., Timmermans, J., van Laake, P., Salama, S., van der Kwast, H., Claassen, E., Stolk, A., Jia, L., Moors, E., Hartogensis, O., and Gillespie, A.: EAGLE 2006 – Multi-purpose, multi-angle and multi-sensor in-situ and airborne campaigns over grassland and forest, Hydrol. Earth Syst. Sci., 13, 833–845, doi:10.5194/hess-13-833-2009, 2009.

Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., and Grennfelt, P. (Eds.): The European Nitrogen Assessment: Sources, Effects and Policy Perspectives, Cambridge University Press, New York, USA, 2011.

Thomas, R. C. and Mead, D. J.: Uptake of nitrogen by *Pinus radiata* and retention within the soil after applying ^{15}N-labelled urea at different frequencies. 2. Seasonal trends and processes, Forest Ecol. Manag., 53, 153–174, 1992.

Vitousek, P. M. and Howarth, R. W.: Nitrogen limitation on land and sea – How can it occur, Biogeochemistry, 13, 87–115, 1991.

Wang, L., Xu, Y. C., and Schjoerring, J. K.: Seasonal variation in ammonia compensation point and nitrogen pools in beech leaves (Fagus sylvatica), Plant Soil, 343, 51–66, 2011.

Xia, J. Y. and Wan, S. Q.: Global response patterns of terrestrial plant species to nitrogen addition, New Phytol., 179, 428–439, 2008.

Yasumura, Y., Onoda, Y., Hikosaka, K., and Hirose, T.: Nitrogen resorption from leaves under different growth irradiance in three deciduous woody species, Plant Ecol., 178, 29–37, 2005.

Zhang, G. L., Jiang, H., Niu, G. D., Liu, X. W., and Peng, S. L.: Simulating the dynamics of. carbon and nitrogen in litter-removed pine forest, Ecol. Modell., 195, 363–376, 2006.

Zhang, L., Wright, L. P., and Asman, W. A. H.: Bi-directional air-surface exchange of atmospheric ammonia: A review of measurements and a development of a big-leaf model for applications in regional-scale air-quality models, J. Geophys. Res., 115, D20310, doi:10.1029/2009JD013589, 2010.